International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 139-142

Received: 01-09-2017
Accepted: 03-10-2017

Kavita T Hegde
Department of Plant Pathology, UAHS, Shivamogga, Karnataka, India

Narayanaswamy H
Department of Plant Pathology, UAHS, Shivamogga, Karnataka, India

Kavitha S Veeraghanti
Department of Plant Pathology, UAHS, Shivamogga, Karnataka, India

Manu TG
Department of Plant Pathology, UAHS, Shivamogga, Karnataka, India

Correspondence
Kavita T Hegde
Department of Plant Pathology, UAHS, Shivamogga, Karnataka, India

Efficacy of bio-agents, botanicals and fungicides against *Fusarium oxysporum* f. sp. *dianthi* causing wilt of carnation

Kavita T Hegde, Narayanaswamy H, Kavitha S Veeraghanti and Manu TG

Abstract
Carnation (*Dianthus caryophyllus* L.) is one of the most important cut flowers in the world. It is one of the major export oriented crop grown in controlled conditions and it is the second most important flower grown in India. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* is one of the major constraints for carnation production causing a yield loss up to 40%. So a total of nine fungicides, six botanicals and two bioagents were screened *in-vitro* against *Fusarium oxysporum* f. sp *dianthi* causing wilt of carnation. Out of which systemic fungicides such as Carbendazim, Propiconazole, Difenconazole were found effective, but the contact fungicide such as Chlorothalonil and Mancozeb were found to be effective at higher concentrations. Among the bioagents *Trichoderma harzianum* (UAHS Shivamogga) isolate was found to be effective than other biogents.

Keywords: *Fusarium oxysporum* f. sp. *dianthi*, fungicides, botanicals, carnation, wilt, bioagents

Introduction
Floriculture is a fast emerging and highly competitive industry. Protected cultivation of flowers gained importance in recent years. In world around 46,008 ha area is under flower production in protected condition. In India, the area of flower crops under protected cultivation is gradually increasing and carnation is one of the most important flower crop grown under protected conditions. Carnation (*Dianthus caryophyllus* L.) is one of the most important cut flowers in the world. It is also known as divine flower, clove pink, gilly flowers etc. The genus name *Dianthus* means flower of zeus or divine. It belongs to the family Caryophyllaceae. Centre of origin for carnation is Mediterranean region.

Cultivation of carnation on commercial scale for domestic and export purpose is relatively recent in India. This crop is being cultivated under polyhouse conditions in Karnataka (Bangalore, Belgaum and Shivamogga), Tamil Nadu (Coimbatore) New Delhi, Maharashtra (Nasik and Pune) Himachal Pradesh (Solan, Simla and Palampur) and Jammu (Srinagar), besides few other places like Uttar Pradesh, Punjab etc.

In India annual production of carnation is 6 MT and 45 per cent production of carnation is from Himachal Pradesh (2.75 MT) followed by Uttarakand 1.25 MT. In Karnataka production of carnation is 0.69 MT (Anon., 2015) [1]. The average yield level per hectare in Karnataka is very low; many factors may be attributed for the low yields, of which one of the important factors may be pests and diseases. Green house condition favours the luxuriant growth of carnation plants. A number of biotic stresses that cause threat to the successful production of Carnation. Among this fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* is one of the major constraints for carnation worldwide. The disease incidence has been reported to be between 40-60 per cent in Germany, Italy and Poland, where as in India around 40 per cent incidence was estimated. In Himachal Pradesh, the pathogen causes huge yield reduction up to 79 per cent in Tropicana cultivar (Katoch, 1999) [2]. Especially in protected cultivation this disease is more severe and it is usually associated with root knot nematode (*Meloidogyne incognita)* causing huge losses to the carnation producers (Nagesh and Reddy, 2001) [3].

Use of fungicides for the control of plant diseases is a common practice. As *Fusarium* is a soil borne pathogen with a wide host range crop rotation may not be of much help. So we have to look for newer fungicides, botanicals and bio-agents to use these in integrated management practises, hence studies were undertaken to evaluate new fungicides, botanicals and bio-agents

to know their efficacy against *Fusarium oxysporum* f. sp. *dianthi* for further utilization in field to manage the disease.

Material and Methods

Isolation of the fungus

The carnation plants showing typical symptoms of Fusarium wilt were collected from naturally ventilated polyhouse and the causal fungus was isolated by adopting the standard tissue isolation technique. Later, the bit of fungal growth was transferred to PDA slants for purification and maintenance of the culture.

Evaluation of bio-agents

Trichoderma harzianum and Pseudomonas fluorescens isolates were tested *in vitro* against *Fusarium oxysporum* f .sp *dianthi* by using dual culture technique (Dennis and Webster, 1971) [4].

Dual culture technique

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of *Fusarium oxysporum* f .sp *dianthi* were inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at one end of the Petri plate to the middle of the Petri plate and the test fungus placed at the other end. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the *Fusarium oxysporum* f .sp *dianthi* and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947) [5].

$$I = \frac{C - T}{C} \times 100$$

Where,
I = per cent inhibition
C = growth in control
T = growth in treatment

Evaluation of botanicals

The poisoned food technique was followed to evaluate the efficacy of botanicals in laboratory against *F. oxysporum* f .sp *dianthi* at concentrations of 2.50, 5.00 and 10.00 per cent with three replications each of different botanicals.

Preparation of plant extracts

Six plants *viz.*, Water hyacinth, Marigold, Neem, Pongamia, Agave and Garlic were selected for the study. Fresh leaves or bulbs of the plants were collected from various locations of UAHS, Shivamogga. These samples were washed thoroughly with tap water and surface sterilized with 1.0 per cent sodium hypochlorite and repeatedly washed with distilled water. Hundred grams of leaf or bulb materials was taken and cut into small pieces, 100 ml water was added and the leaf materials were crushed using a grinder. The stock solution of all the leaf extracts was collected by filtering with muslin cloth.

To study the antifungal mechanism of plant extracts the poisoned food technique was used (Nene and Thapliyal, 1982) [6]. Two, five and ten ml of stock solution was mixed with 97.5, 95 and 90 ml of sterilized molten PDA media respectively so as to get 2.5, 5.0 and 10.0 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract.

The medium was poured into petri plates. These plates were inoculated with 5 mm disc of freshly grown cultures of *F. oxysporum* f. sp *dianthi*. Suitable control plates were maintained where in culture discs were inoculated into the center of potato dextrose agar plates without plant extracts. Radial growth of the fungus was measured after seven days of inoculation and the per cent inhibition of growth of the test fungus was calculated by using the formula given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,
I = per cent inhibition
C = growth in control
T = growth in treatment

Evaluation of Fungicides

The efficacy of non-systemic fungicides and systemic fungicides against *Fusarium oxysporum* f. sp. *dianthi* were assessed by following poisoned food technique as explained above. Here the required quantities of individual fungicides were added separately into molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides and the remaining procedures were similar to plant extract evaluation. The per cent inhibition of growth of the test fungus was calculated by using the formula given by Vincent (1947).

Results and Discussion

Bio-agents

Among the different bio-agents tested, maximum reduction in colony growth was observed in *T. harzianum* (UAHS isolate) (64.44%) which is on par with *T. harzianum* (UASD isolate) (62.91%). Least reduction of colony growth was observed in *Pseudomonas fluorescens* (UAHS) (47.74%) (Table 1).

These results were supported by Mahalaxmi and Yesu Raja (2013) [7] and Pratibha Sharma (2000) [8] who reported the efficacy of *T. harzianum* against *F. oxysporum* f. sp. *dianthi*. This inhibition may be due to volatile and non-volatile metabolites and cell wall degrading enzymes produced by *Trichoderma* spp.

Table 1: *In-vitro* evaluation of bio-agents against *F. oxysporum* f. sp. *Dianthi.*

S. No.	Bio-agents	Per cent inhibition
1	*Trichoderma harzianum* (UAHS)	64.44 (53.42)
2	*Trichoderma harzianum* (GKVK)	59.57 (50.52)
3	*Trichoderma harzianum* (UASD)	62.91 (52.49)
4	*Pseudomonas flouroscens* (UAHS)	47.74 (43.71)
5	*Pseudomonas flouroscens* (UASD)	60.55 (51.14)
	Mean	55.38 (50.25)
	SEm±	0.90
	CD at 1%	2.72

* Figures in parentheses are arcsine transformed values

Plant extracts

The antifungal activity of six plant extracts were assayed, for their efficacy against the *F. oxysporum* f. sp. *dianthi* using

poisoned food technique. The results revealed that, effect of plant extracts on the fungal growth was significant. Garlic extract (89.44%) was found effective in inhibiting mycelial growth which was superior over all other plant extracts and it was followed by neem leaf extracts (68.32%) and pongamia extract (62.58%). Least inhibition was observed in agave (33.07%) followed by marigold (41.84%).

Interactions between botanicals and concentrations were significant. All the plant extracts reduced the mycelial growth with increase in concentration. The various leaf extract at 10 per cent concentration was significantly superior over 5.00 per cent and 2.50 per cent. Garlic extract at 10 per cent (91.11%) was found effective in inhibiting mycelial growth which was on par with garlic extract at 5% (90.55%) and neem extract at 5% (90.55%). Least inhibition was recorded in agave (15.55%) and marigold leaf extracts (20.55%) at 2.5% per cent (table 2).

These results were in agreement with the findings of Kishore, 2007 [9], who reported among clove oil and garlic extract found to be most effective against *F. oxysporum* f. sp. *dianthi*. The inhibitive action of garlic bulb crude extract on fungal growth has been attributed to the existence of allicin, as the major anti-bacterial, anti-fungal and anti-viral component (Miron *et al.,* 2000) [10]. The fungicidal spectrum of *Azadirachta indica* has been attributed to presence of azadiractin which belongs to C25 terpenoides compounds (Subramanian 1993) [11].

Table 2: *In-vitro* evaluation of botanicals against *F. oxysporum* f. sp. *Dianthi.*

Sl No.	Botanicals	Per cent inhibition Concentrations (%)			Mean
		2.5	5	10	
1	Pongamia (*Pongamia pinnata*)	49.41 (43.49)	66.11 (53.63)	72.22 (57.26)	62.58(51.46)
2	Garlic (*Allium sativum* L.)	86.66 (67.41)	90.55 (71.96)	91.11 (73.05)	89.44 (70.80)
3	Neem (*Azadirachta indica*)	44.99 (42.86)	69.44 (56.35)	90.55 (72.16)	68.32 (57.12)
4	Agave (*Agave americana*)	15.55 (23.43)	35.33 (36.45)	48.33 (43.72)	33.07 (34.53)
5	Water hycianth (*Eichhornia crassipes*)	53.33 (48.85)	47.22 (43.72)	60.55 (51.63)	53.70 (48.07)
6	Marigold (*Tagetes erecta*)	20.55 (26.24)	48.88 (42.65)	56.10 (48.61)	41.84 (39.17)

	S.Em±	CD at 1 %
Botanicals (B)	0.60	2.32
Concentration (C)	0.85	3.28
B x C	1.48	5.19

* Figures in parentheses are arc sine transformed values

Fungicides

Four systemic, four non systemic fungicides and one combi product were screened at four concentrations for their efficacy against *F. oxysporum* f. sp. *dianthi by* poison food technique. In contact fungicides, Chlorothalonil (82.40%) gave maximum inhibition of mycelial growth, which was superior over all other contact fungicides which was followed by Mancozeb (61.29%). Least inhibition of mycelial growth was observed in Copper oxy-chloride (45.83%) (Table 3).

Among the different contact fungicides and concentration tested, Chlorothalonil at 1500 ppm (87.40%) concentration gave maximum inhibition of the mycelial growth of pathogen, which was on par with Chlorothalonil at 1000 ppm (85.92 %) and Mancozeb at 1500 ppm (85.55%) which was followed by Chlorothalonil at 500 ppm (83.70%). Least inhibition was in Copper oxychloride at 250 ppm (18.51%) and Mancozeb at 250 ppm (29.62%).

These results were in accordance with results of Kishore, 2007 [9], found maximum inhibition of *F. oxysporum* f. sp. *dianthi* by Mancozeb and Chlorothalonil whereas copper oxy-chloride was least effective.

There was a significant difference among the systemic fungicides in inhibiting the growth of *F. oxysporum* f. sp. *dianthi*. Among the four systemic fungicides and one combi product evaluated, Carbendazim (91.99%) was significantly superior over other treatments, followed by Propiconazole (87.95%) and Difenoconazole (83.79%). Least inhibition was observed in fungicide, Mancozeb 63% +Carbendazim 12% (52.63%).

Among the different concentrations of various fungicides tested, 1000 ppm (83.95 %) was very effective followed by 500 ppm (78.54 %). Least inhibition was observed in 100 ppm concentrations (71.97 %) of various fungicides.

Carbendazim at 1000 ppm (94.81%) was very effective and was on par with Carbendazim at 500 ppm (94.07%), Propiconazole at 1000 ppm (92.58%) and Carbendazim at 250ppm (91.66%). Least inhibition of mycelial growth of pathogen was observed in fungicide, Mancozeb 63% +Carbendazim 12% (41.48 %) at 100 ppm concentration (Table 4).

Table 3: *In-vitro* evaluation of contact fungicides against *F. oxysporum* f. sp. *Dianthi.*

S. No	Fungicides	Trade name	Per cent inhibition (%) Concentration (ppm)				Mean
			250	500	1000	1500	
1	Copper oxychloride 50WP	Blitox	18.51 (25.28)	35.18 (36.36)	54.44 (47.55)	75.18 (60.19)	45.83 (42.35)
2	Captan 50WP	Captaf	50.73 (45.42)	52.18 (46.25)	58.14 (49.68)	65.18 (53.83)	56.56 (48.80)
3	Mancozeb	Indofil M 45	29.62 (32.96)	50.73 (45.42)	79.25 (62.92)	85.55 (67.69)	61.29 (52.25)
4	Chlorothalonil 75WP	Kavach	72.21 (58.21)	83.70 (66.21)	85.92 (67.97)	87.40 (69.28)	82.40 (65.49)
Mean			42.76 (40.46)	55.44 (48.56)	69.43 (61.52)	78.33 (62.75)	

*Figures in parentheses are arc sine transformed values

	S. Em±	CD at 1 %
Fungicide (F)	0.59	1.79
Concentration (c)	0.59	1.79
F x C	1.19	3.68

Table 4: *In-vitro* evaluation of systemic fungicides against *F. oxysporum* f. sp. *Dianthi.*

S. No	Fungicides	Trade name	Per cent inhibition Concentration (ppm)				Mean
			100	250	500	1000	
1	Propiconazole 25EC	Tilt	81.29 (64.52)*	88.51 (70.31)	89.44 (71.08)	92.58 (74.23)	87.95 (70.04)
2	Mancozeb 63% + Carbendazim 12% 75WP	Saaf	41.48 (40.06)	49.07 (44.46)	50.91 (45.52)	69.07 (56.22)	52.63 (46.56)
3	Difenconazole 25EC	Score	80.74 (64.07)	82.77 (65.65)	85.55 (67.69)	86.10 (68.13)	83.79 (66.38)
4	Thiophanate methyl 75WA	Roko	67.46 (55.24)	69.07 (56.28)	72.77 (55.86)	77.21 (58.13)	71.62 (56.37)
5	Carbendazim 50 WP	Bavistin	88.88 (70.53)	91.66 (73.39)	94.07 (75.91)	94.81 (76.85)	92.36 (74.17)
Mean			71.97 (58.88)	76.21 (62.02)	78.54 (63.21)	83.95 (66.71)	

*Figures in parentheses are arc sine transformations

	S.Em±	CD at 1 %
Fungicide (F)	0.82	2.53
Concentration (C)	0.73	2.87
F x C	1.64	4.92

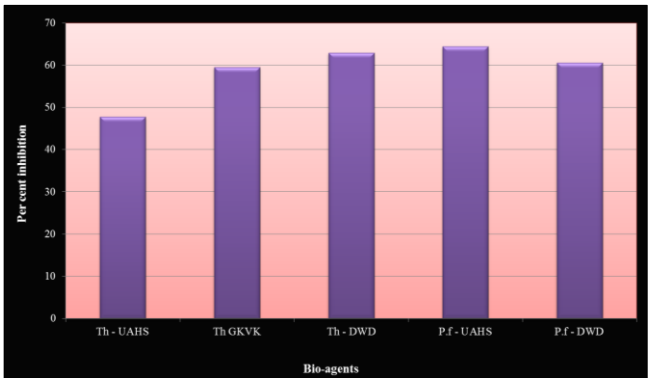

Fig 1: *In-vitro* evaluation of bio-agents against *F. oxysporum* f. sp. *dianthi.*

The results obtained were in confirmity with the observation made by Sunita and Katoch (2001) [12], who reported that carbendazim and benomyl which completely inhibited the rowth of the test fungus. Similarly Kulkarni (2006) [13], reported that Carbendazim was highly effective in inhibiting growth of *F. oxysporum* f. sp. *gladioli* and Kishore (2007) [9] reported that Carbendazim was superior over thiophanate methyl. This inhibition was mainly due to the action of fungicides on particular functions of the fungus and the triazole fungicides were found to be effective in the present study as they act by inhibiting the demethylation step in the biosynthesis of sterol, which is need in fungal cell walls, they most likely bind to cytochrome P-450 involved in sterol demethylation (Uesugi, 1998) [14].

References

1. Anonymous. Indian Horticulture Database, NHB, Ministry of commerce, Govt. of India, 2015, 286.
2. Katoch R. Studies on Fusarium wilt of carnation. *M.Sc. thesis*, Dr. Y.S. Parmar Univ. of Horti, Forestry, Solan, 1999, 53.
3. Nagesh M, Parvatha Reddy PN. Pathogenicity of selected antagonistic soil fungi egg masses under *in* vitro and in vivo conditions. J. Biological Control, 2001; 15:63-68.
4. Dennis C, Webster J. Antagonistic properties of species groups of Trichoderma II. Production of volatile antibiotics. Trans. British Mycol. Soc. 1971; 57:41-48.
5. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 1947; **150**:850.
6. Nene YL, Thapliyal PN. Fungicide in Plant Diseases Control, III Edition: Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, 1982, 325.
7. Mahalakshmi P, Yesu Raja I. Biocontrol potential of *Trichoderma* species against wilt disease of carnation *Dianthus caryophyllus* L. caused by Fusarium oxysporum f. sp. dianthi. J of Biopest. 2013; 6(1):32-36.
8. Pratibha Sharma. An integrated approach for the management of carnation wilt caused by *Fusarium oxyspor*um f. sp. dianthi Pril. and Del. Snyd. and Hans. New Botanist. 2000; 27:143-150.
9. Kishore C. Studies on diagnosis and management of fungal wilt diseases of carnation and gerbera under protected cultivation. M. Sc. Agri thesis, Univ. Agric. Sci., Dharwad, Karnataka, India, 2007, 84.
10. Miron TA, Rabinikov D, Mirelman M, Wilchek, Weiner L. The mode of action of allicin: Its ready permeability through phospholipid membranes may contribute to its biological activity. Biochinica et Biophysica Acta., 2000; 1463:20-30.
11. Subramanian P. Effect of plant extracts in controlling fungal disease of groundnut. *Oilseeds J.* 1993; 10:67-69.
12. Sunita Chandel S, Katoch R. Chemical control of Fusarium oxysporum f. sp. dianthi, an incitant of carnation wilt. Indian J Microbiol. 2001; 41:135-137.
13. Kulkarni Sumitra P. Studies on *Fusarium oxysporum* Schlecht Fr f. sp. gladioli Massey Snyd. & Hans. causing wilt of Gladiolus. M. Sc. Agri. thesis, Univ. Agric. Sci. Dharwad India, 2006, 42-76.
14. Uesugi Y. Fungicide classes: chemistry, uses and mode of action. In: Huston, D., Miyamoto, J Eds., Fungicidal Activity-Chemical and Biological Approaches to Plant Protection. Wiley, Chichester, England, 1998, 23-56.